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EFFECT OF LEUKOTRIENES C_4 AND D_4 ON PROSTAGLANDIN I_2-LIBERATION FROM HUMAN LYMPHATICS

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ABSTRACT

Whereas prostaglandin I_2 (PGI_2), a major metabolite of human lymphatics, does not itself affect lymphatic contractility significantly, it is able to counterbalance the contractile response to thromboxane and leukotrienes. We now demonstrate that leukotrienes C_4 and D_4 evoke a dose-dependent increased production of PGI_2 from human lymphatics. It is likely that leukotrienes either exert a contractile rhythmic effect on human lymphatics or, alternatively, evoke increased PGI_2-formation which relaxes human lymphatics. These mechanisms may be of local importance in regulating lymphatic "tone" at sites of inflammation as leukotrienes are liberated from activated white blood cells.

Rhythmic contraction of human lymphatics has been recognized for centuries (1) and is now considered a major factor in lymph propulsion (2). Olszewski and Engeset (3) first pointed out that prostaglandin F_2-alpha induced contraction of lymphatics. Later Johnston et al (4,5) observed that thromboxane A_2 stimulated lymphatics to contract and leukotrienes exerted a rhythmical contraction of lymphatics. These findings, in conjunction with evidence that various prostaglandins are found in the effluent of lymph (6-8), raised the question whether stimulation of PGI_2-synthesis by leukotrienes C_4 and D_4 (7,8) occurred in human lymphatics, and whether contraction of lymphatics was rhythmically coregulated by these eicosanoids (4,9).

MATERIALS AND METHODS

We studied three human lymphatics (obtained during lymphangiography after informed consent) from two males and one female aged 15-41 years. These lymph vessels were cut into rings with a circumference of approximately 0.5 cm. Two wires were fixed to the lumen as described by Johnston and Gordon (10), the lower one fixed to the bottom of a 5 ml perfusion bath, the upper one connected with an isometric transducer (Harvard Instruments recorder Pharmacia). Lymphatics were constantly perfused with an oxygenated (95% O_2, 5% CO_2) Krebs-Ringer solution at a constant temperature of 37°C. Thereafter, the lymphatics were placed under 0.5g. The leukotriene was added in concentrations of $2x10^{-8}$, $2x10^{-7}$, $2x10^{-6}$.

From five lymph vessels of three males and two females in the age range of 16-47 years PGI_2-formation (1) was bioassayed. Briefly, tissue samples were incubated at 22°C for three minutes in Tris HCL buffer (pH 7.4). After incubation, 100 µl of the incubation buffer were removed and added to a platelet rich-plasma. Platelet-rich plasma was prepared after anticoagulation with 3.8% sodium citrate and adjusted to a con-

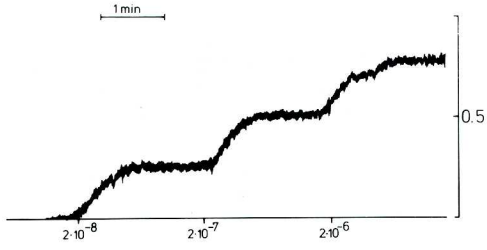

Fig. 1: Greater lymphatic contractile response to incremental higher doses of leukotriene C_4.

stant platelet count of $250x10^3/\mu l$. In the prewarmed aggregometer one minute later aggregation was induced by ADP (100 µl, 1µmol). The inhibitory activity of the incubation buffer was quantified using a synthetic PGI_2 standard. Together with the tissue samples the leukotrienes C_4 and D_4 were incubated in a dose range from one to 100 ng/ml. Prostacyclin formation is shown in pg/mg/min.

STATISTICS

Values are shown as mean ± SD; calculation for significance was done using Student's t-test.

RESULTS

Leukotriene C_4 caused a rhythmic contraction of human lymph vessels (*Fig. 1*). Leukotrienes C_4 and D_4 promoted a dose-dependent increase in prostaglandin I_2-formation by human lymphatics reaching the level of significance at doses $>$50ng/ml (*Table 1*).

Table 1.
Stimulation of PGI_2-synthesis by LTC_4 and LTD_4
($\bar{x}$ ± SD)

Amount	n	LTC_4	LTD_4
buffer	4	4.71±2.27	4.86±2.51
+ 1 ng	4	4.85±2.35	4.80±2.43
+ 5 ng	4	4.78±2.17	4.93±1.83
+ 10 ng	4	5.58±2.29	5.17±1.96
+ 50 ng	4	7.63±2.71*	8.04±2.57*
+ 100 ng	4	10.84±2.16*	11.23±2.27*

* $p < 0.01$

DISCUSSION

Leukotrienes are formed predominantly by white blood cells (12) and induce a notable increase in prostaglandin I_2-formation (7,8) via prostacyclin synthetase. Normally, human lymphatics are not able to synthesize leukotrienes (13), and it is likely that as part of the local inflammatory response white blood cells are the primary source of these eicosanoids. The double action of leukotrienes (i.e. either inducing muscular contraction directly or indirectly promoting relaxation by counteracting contractions due to PGI_2-synthesis induced by leukotrienes) may be a key regulator of human lymphatic contractility, especially during an inflammatory response. Many other factors, such as acid pH (causes a shortened half-life of biologically active prostacyclin) or varying concentrations of albumin and other proteins (12) modulate the biological half-life and thus bioavailability of these compounds. In brief, the rhythmic contraction of lymphatics evoked by leukotrienes, or lymphatic relaxation induced by enhanced PGI_2-formation suggest that eicosanoids are important regulators of lymphatic motility particularly where white blood cells accumulate (e.g. sites of inflammation).

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